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Full Length Research Paper

In silico effective inhibition of galtifloxacin on built Mtb-DNA gyrase

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Tuberculosis (TB) resurged in the late 1980s and now kills approximately 3 million people a year. The reemergence of tuberculosis as a public health threat has created a need to develop new anti-mycobacterial agents. The Mtb-DNA Gyrase is an attractive target for development of new drugs due to its indispensable role in catalyzing the negative supercoiling of DNA and is essential for efficient DNA replication, transcription and recombination. Fluoroquinolone families of inhibitors are developed against the Mtb-DNA gyrase which show the best inhibition with DNA gyrase in the past. Due to the development of Multi-drug resistant Mycobacterium tuberculosis strains, the drugs showed less efficiency on the targets, recently, a new flouroquinolone inhibitor was identified (Galtifloxacin), which shown best inhibition. In this study we carried out Homology model of 0 Mtb-DNA gyrase, secondary structure analysis and active site analysis. Docking studies were also carried out with the Galtifloxacin and Amifloxacin and are helpful for further studies on the development of novel drugs against Mtb-DNA gyrase.

Key words: Mycobacterium tuberculosis, DNA gyrase, galtifloxacin, amifloxacin, homology modeling, docking

INTRODUCTION

Tuberculosis (TB) is a contagious and deadly disease that spreads through the air, which has reached pandemic proportions. According to the World Health Organisation (WHO), in 2006 there were 9.2 million new cases and 1.7 million deaths from TB around the world (http://www.who.int/tb/en/). A significant proportion of these new cases and deaths occur in HIV-positive people. Owing to population growth, the number of new cases arising each year is increasing globally, posing a continued health and financial burden in various parts of the world, particularly Asia and Africa. TB is caused predominantly by Mycobacterium tuberculosis (Mtb), an obligate aerobic bacillum that divides at an extremely slow rate. The chemical composition of its cell wall includes peptidoglycans and complex lipids, in particular mycolic acids, which are a significant determinant of its virulence (Shah et al., 2007; Sylvain et al., 2007). The unique structure of the cell wall of Mtb allows it to lie dormant for many years as a latent infection, particularly as it can grow readily inside macrophages, hiding it from the host's immune system. The vast majority of TB infections are caused by Mtb, but other closely related mycobacteria (*Mycobacterium bovis* and *Mycobacterium africanum*) can also cause the disease (Ducati et al., 2006; Morcillo et al., 2007). When TB becomes active, it typically affects the lungs (pulmonary TB), but in around 25% of cases (immunosuppressed persons and young children), the bacteria enter the blood and infect other parts of the body, such as the pleura, the meninges, the lymphatic system, the genitourinary system and the bones and joints. Infection with HIV suppresses the immune system, rendering individuals more susceptible to TB infection, or allowing a latent infection to activate (Rosas-Taraco et al., 2006). Co-infection allows faster progress of both TB and HIV (Goletti et al., 1996) and also uncontrolled treatment, with rifampicin and isonizaid however, has led to rise of multi-drug-resistant TB incidence (MDR-TB: resistance to isonizaid and rifampicin and possibly other drugs), Poor compliance to the therapy

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using these drugs combined with the second-line anti-tuberculotic (e.g. Pyrazinamide and ethambutol) resulted in extremely drug-resistant strains (XDR-TB: resistant to at least three of the available antituberculotics including rifampicin and isoniazid) (Babajan et al., 2009). The emergence of such strain urges the development of novel drug targets and drugs.

Most well known bacterial drug targets are the type II DNA topoisomerases, DNA Gyrase and topoisomerase IV. These ATP-dependent enzymes act by a transient double-stranded DNA break and cooperate to facilitate DNA replication and other key DNA transactions (Levine et al., 1998). DNA Gyrase is unique in catalyzing the negative supercoiling of DNA and is essential for efficient DNA replication, transcription and recombination, whereas topoisomerase IV has a specialized role in chromosome segregation. DNA Gyrase is a tetrameric A2B2 protein. The A subunit (90 to 100 kDa, depending on the bacterial species) carries the breakage-reunion active site, whereas the B subunit promotes ATP hydrolysis, needed for energy transduction. Mycobacterium tuberculosis genes encoding DNA Gyrase were identified from the genome analysis as a gyrB-gyrA contig in which gyrA and gyrB encode the A and B subunits, respectively (Madhusudan et al., 1994). Surprisingly, there is no evidence of the topoisomerase IV parC and parE gene homologs in the genome of Mycobacterium tuberculosis (Cole et al., 1998). It appears that DNA Gyrase is the sole topoisomerase drug target in Mycobacterium tuberculosis. The absence of a homologue in eukaryotic cells makes Mtb-DNA Gyrase an attractive target for small molecule inhibitors with the potential to have broad antibacterial activity. The Fluoroquinolone are a family of synthetic broad-spectrum antibiotics. They prevent bacterial DNA from unwinding and duplicating (Hooper et al., 2001). Since bacteria and humans unwind DNA with different enzymes, most of those enzymes (topoisomerases) in humans are not affected.

To the best of our knowledge, little attention has been paid to the theoretical study on the three-dimensional modeling of Mtb-DNA Gyrase to make a deeper understanding of Mtb-DNA Gyrase at molecular level, an attempt is made in this paper to build up a three-dimensional model of Mtb-MurB by the homology module of mod9v5 and structural characterization performed using different *in silico* approaches.

MATERIALS AND METHODS

All calculations were conducted on AMD-Athlon 64 bit, 3.4MHz, Dual processing machine. *In silico* analysis of the Mtb-DNA Gyrase structural model proceeded in three steps: sequence analysis, homology modeling and inhibitors docking and scoring. The procedures employed for each step are described below.

Molecular modeling

The amino acid sequence (15607148) of Mtb-DNA Gyrase was obtained from National Center for Biotechnology Information (NCBI, http:// www.ncbi.nlm.nih.gov/). Homologous sequence identity was carrier out through the BLASTp server (Altschul et al., 1990), to search for short nearly exact matches and corrected the dataset to remove redundant sequences. Multiple sequence alignments for a series of Mtb-DNA Gyrase, were conducted using the ClustalW1.8 routine with default parameters (Chenna et al., 2003). They were grouped them based on sequence source (6 different sources). The alignment revealed functionally important conserved residues in all DNA Gyrase family of enzymes. Search of the PDB database using the sequence of Mtb-DNa Gyrase as the entry, structures of *Escherichia coli* DNA Gyrase (PDB code 1AB4) and *Staphylococcus aureus* topoisomerasae IV (PDB code 2INR) were used as templates to build a 3D model of Mtb-DNA Gyrase. A structural model of Mtb-DNA Gyrase was generated through homology modeling by the program Modeller9v5 (Sali et al., 2003). Total of 100 structures were generated, from which best one model were checked followed by optimization. Optimization of protein reduces the steric clashes of the side chains without modifying the backbone of the protein and it was able to solve bad contacts. And finally 1AB4, 2INR and Mtb-DNA Gyrase models were checked to assess the quality of the structure, resulting in the PROCHECK (Laskowski et al., 1993). To predict the secondary structure and active site amino acids of Mtb-DNA Gyrase 3D model was submitted to PDBSUM server (Laskowski et al., 2005).

Mtb-DNA gyrase docking with major fluoroquinolone inhibitors

For Docking with Autodock4, Galtifloxacin and Amifloxacin inhibitors were retrieved from pubchem (CID:5379, CID:55492) and were optimized using hyperchem program and AutoTors, as implemented in the Autodock tool kit (ADT) software program (Osterberg et al., 2002), which was used to define the torsional degrees of freedom to be considered during the docking process. The number of flexible torsions defined for L-CA and its derivative, was nineteen. Preparation of Mtb-DNA Gyrase enzyme with the AutoDock Tools software involved the addition of polar hydrogen atoms to the macromolecule, a necessary step for the correct calculation of partial atomic charges. Gasteiger charges were calculated for each atom of the macromolecule in AutoDock 4.0 instead of Kollman charges, which were used in the previous versions of this program. Histidine residues were maintained unprotonated as previously determined to be appropriate three-dimensional affinity grids of size 50 × 50 × 50 Å with 0.375 Å spacing were positioned around the active site. For every snapshot of protein, the center of the grid was set to the position of the neighborhood of the ASP145 cavity using the average coordinates of Cα atoms of Arg150 and Tyr206. During each docking experiment, 50 runs were carried out. The rest of the parameters were set as default values. At the end of a docking experiment with multiple runs, a cluster analysis was performed. Docking solutions with a inhibitors all-atom root mean square deviation (RMSD) within 0.1 nm of each other were clustered together and ranked by the lowest docking energy.

RESULTS AND DISCUSSION

3D model building

Figure 1 explains final alignment, which was modeled as Mtb-DNA Gyrase in Modeller 9v5. This alignment was obtained after manual adjustments of the initial alignment from the BLAST server. Four reference proteins, PDB, ID, 1AB4 and 2INR were used to model the structure of the Mtb-DNA Gyrase and homology scores comparing to target proteins were 60 and 59%, respectively. High level

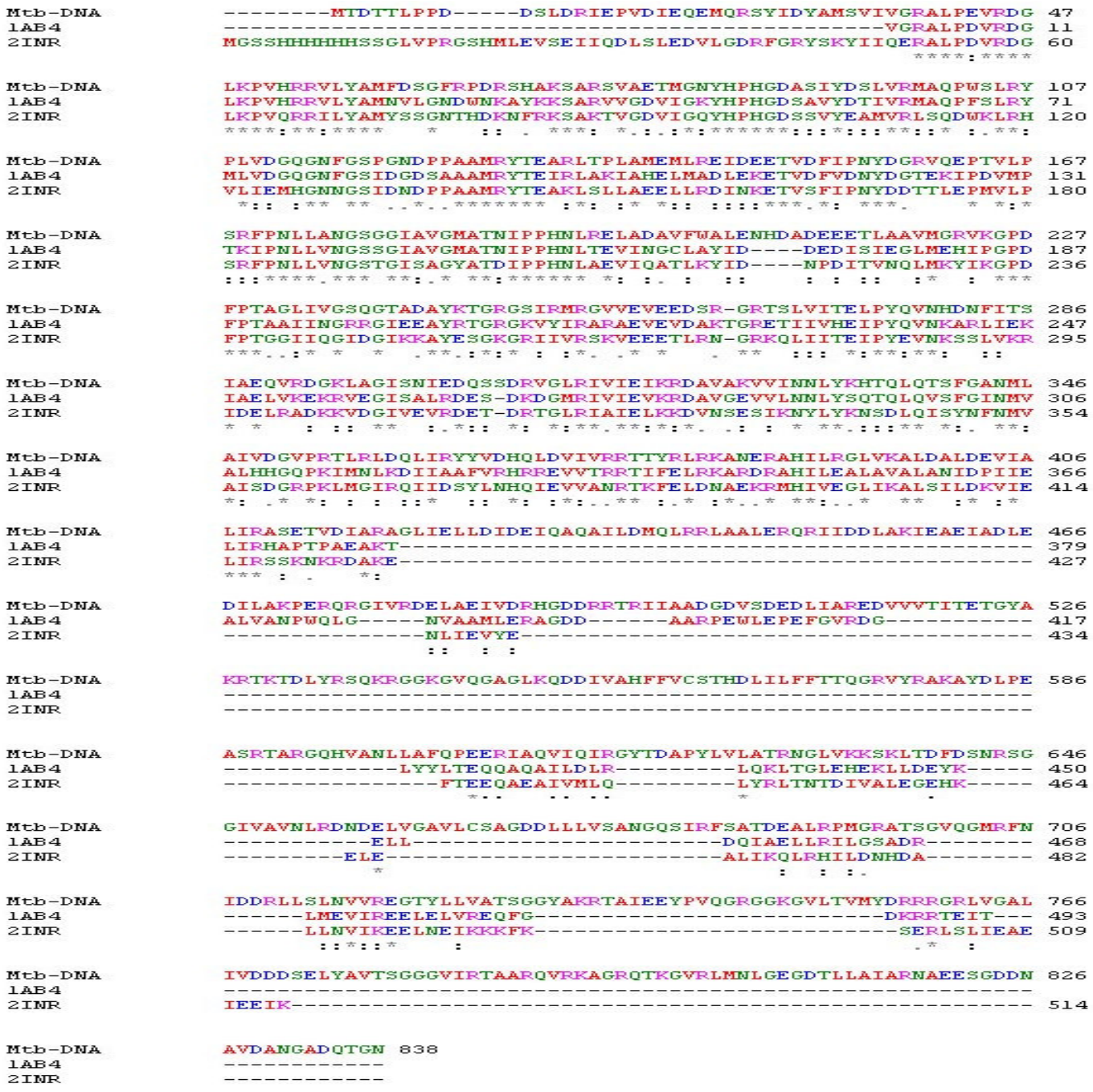

Figure 1. Multiple sequence alignment of Mtb-DNA gyrase with crystal structure of *E. coli* topoisomerase (1AB4) and with topoisomerase of Streptococcus (2INR) the gap (-) represent the deleted regions, star (*) represents the conserved regions in the Figure.

of sequence identity could guarantee more accurate alignment between the target sequence and template structure. In order to define structurally conserved regions (SCRs) of the protein family, the multi-dimensional alignment based on the structural identity was used to superimpose four reference structures and 126 SCRs were determined (Figure 1). The Modeller program uses the spatial constraints, determined from the crystal structure of a template protein, to build a 3D model of the target protein with unknown tertiary structures, on the basis of amino acid sequence homology to the sequence alignment (Figure 2a). The Ramachandran plots reveal more than 95% of the amino acid residues in the favorable regions of the plot for the whole enzyme. The

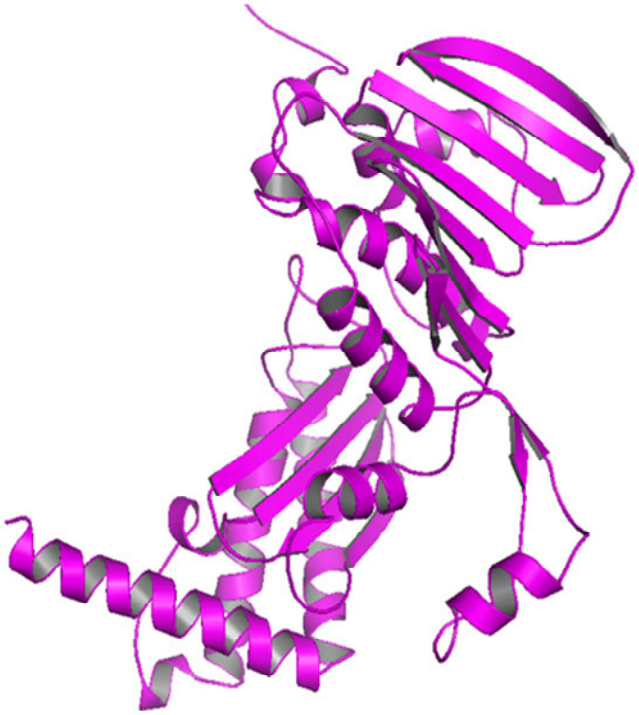

Figure 2a. Built 3 D model of Mtb-DNA Gyrase.

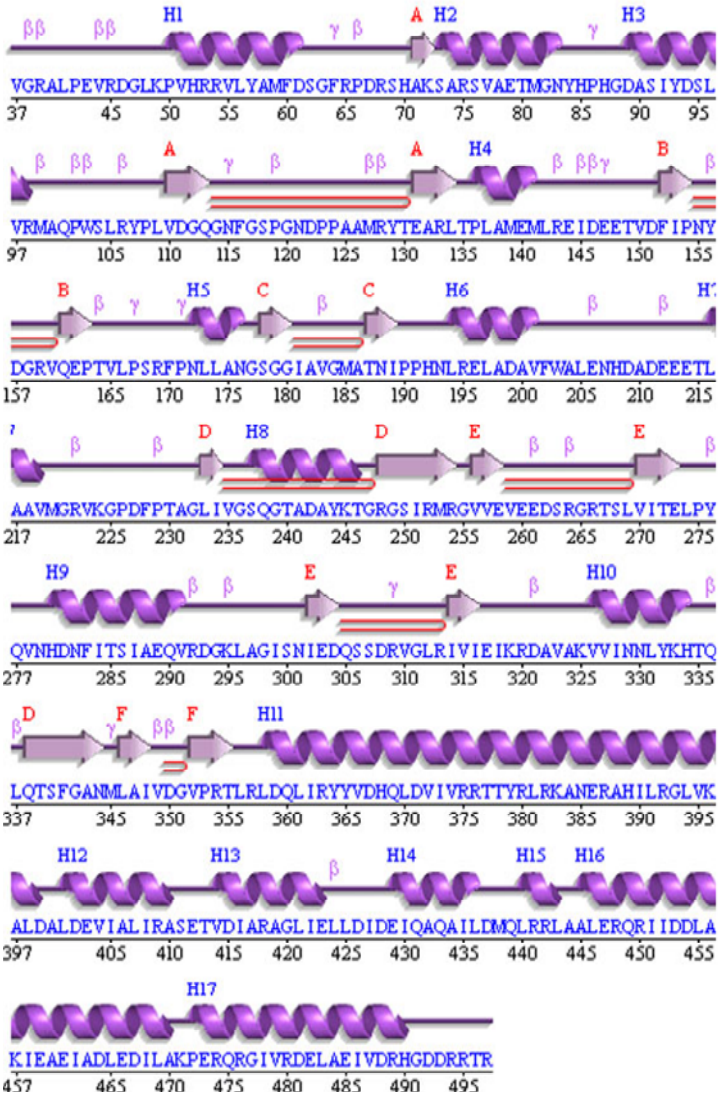

Figure 2b. Secondary structure of Mtb-DNA Gyrase.

main structural elements of the optimized Mtb-SHMT homology model as appeared are shown in Figure 3. In secondary structure, build model consists of three domains, the N-terminal domain, the second N-terminal domain and small domain, The N-terminal domain mediates inter subunit contacts and folds into two α-helices and one β-stand. The second N-terminal domain or large domain binds PLP, has most of the active site residues and folds into an α-β-α sandwich containing nine confirmations. The confirmation clearly appears as four beta sheets, three beta-alpha-beta motifs, four beta hairpins, one beta bulge, fifteen stands, , twenty two helices, thirty five helix-helix interactions, nineteen beta turns and three gamma turns (Figure 2b).

Active site analysis

The active site amino acids residues in built Mtb-DNA Gyrase model was accomplished based on its alignment to the templates 1AB4 and 2INR, which are shows 3 residues that is Arg65, Tyr100 and Asp160 (Figure 4a and 4b). Super position of the templates 1AB4 and 2INR on to Mtb-DNA Gyrase model shows structural similarity at actives site residues.

Inhibitors docking with Mtb-DNA gyrase

Most docked inhibitors interacted by the same mode of the inhibitors, Galtifloxacin and Amifloxacin within the Mtb-DNA Gyrase binding site. The different surface pocket for residue seems to be an important factor in determining the different mode of Galtifloxacin interaction with Mtb-DNA Gyrase of Arg^{65} and Asp^{160} amino acid residues (Figure 4a). Where the Amifloxacin shown interaction with amino acids Tyr^{100} and Arg^{65} (Figure 4b).

The energies of these residues were calculated based on their best docking scores (Table 1), that showed the binding free energies for Galtifloxin was -15.28 kcal/mol, RMSD of 0.08, inhibitory constant of $+1.43e^{-13}$ for rank one cluster. For Amifloxacin binding energies was -11.08 Kcal/mol, RMSD of 0.25, inhibitory constant of $+4.87e^{-8}$ for rank one cluster. It is revealed that energy difference and cluster runs of the Galtifloxacin, Amifloxacin with Mtb-DNA Gyrase, Galtifloxacin shown best interaction compare with Amifloxacin.

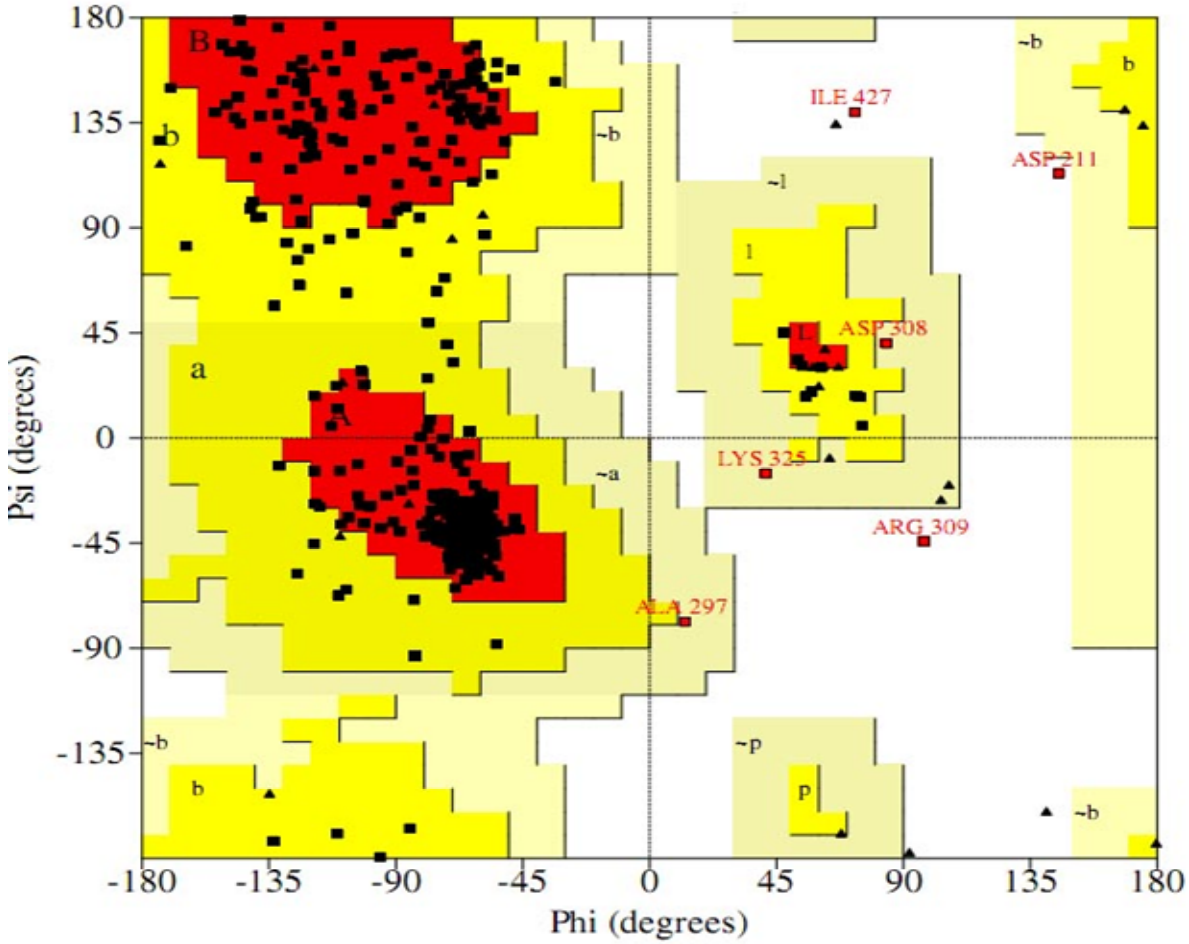

Figure 3. Built Mtb-DNA Gyrase Ramachandran plot.

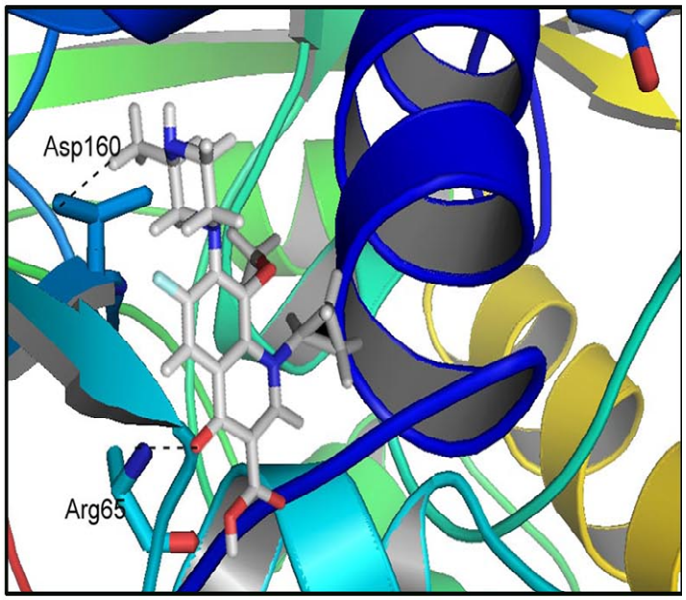

Figure 4a. Galtifloxin interaction with active site amino acids Mtb-DNA Gyrase Asp160 and Arg65.

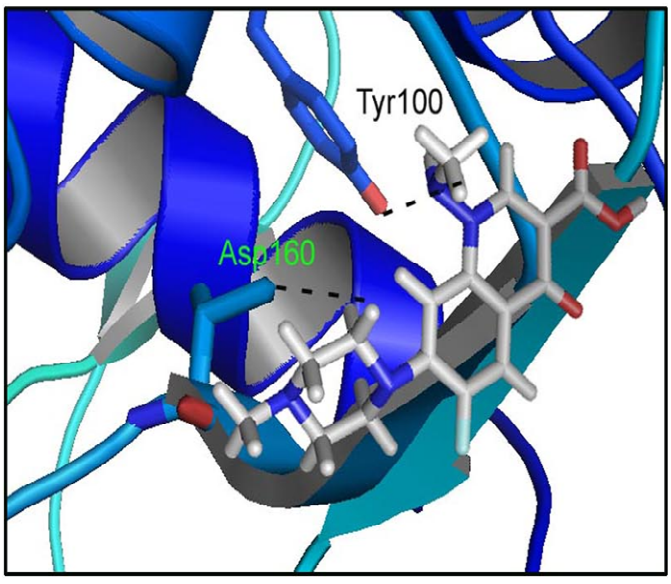

Figure 4b. Amifloxacin interaction with active site amino acids of Mtb-DNA Gyrase Asp160, Tyr100.

Table 1. Docking score of Galtifloxacin and Amifloxacin with Mtb-DNA Gyrase.

Protein	Ligand	Cluster rank	RMSD	Lowest energy (Kcal/mol)	Free energy (ΔG)	Inhibition constant *Ki*
Mtb-DNA gyrase	Galtifloxacin	1	0.08	-15.28	-15.33	$+1.43e^{-08}$
		2	1.25	-11.03	-11.16	$+3.02e^{-08}$
		3	0.23	-10.78	-10.89	$+5.45e^{-08}$
		4	0.00	-10.43	-10.52	$+7.55e^{-08}$
	Amifloxacin	1	0.25	-11.08	-11.20	$+4.87e^{-08}$

Conclusion

In this study, we performed the homology modeling of Mtb-DNA Gyrase through the sequence similarity of 60% and we have adopted a stringent to measure the Mtb-DNA Gyrase enzyme which is not homologous to human (negligible similarity above the e-value threshold of 0.005). We also use the docking studies on Mtb-DNA Gyrase with currently market available drugs; Galtifloxacin and Amifloxacin. The docking studies explore the opportunities opened by the differences found for the interactions of Galtifloxacin and Amifloxacin and it was shown that the Galtifloxacin is a very good target for Mtb-DNA Gyrase compared to Amifloxacin. The results were satisfactory for development of more accurate Galtifloxacin derivative drugs against deadly Mycobacterium.

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